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F. Zhang[a]
R.F. Gagnon[a]
G.K. Richards[b]
A.G. Brox[c]

[a] Department of Medicine, Montreal General Hospital, Montreal, Que.,
[b] Department of Medicine, South Saskatchewan Hospital Center, Regina, Sask., and
[c] Department of Medicine, Royal Victoria Hospital, Montreal, Que., Canada

Anemia of Chronic Renal Failure: Characterization in the Mouse and Correction with Human Recombinant Erythropoietin

Key Words
Chronic renal failure
Anemia
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Mouse

Abstract
Anemia is a cardinal feature of chronic renal failure (CRF) which contributes significantly to the clinical syndrome of chronic uremia. We have conducted a detailed examination of the hematological changes in CRF in the inbred mouse strain C57BL/6J. As in the human situation, CRF mice presented major hematological changes affecting primarily the erythroid cell series. Despite the presence of abundant iron stores in the bone marrow, the CRF mice developed a hypoproliferative anemia of a severity commensurate with the degree of renal impairment. The levels of circulating erythropoietin (EPO) in CRF mice were not significantly different from those in normal control littermates and were therefore inappropriately low for the degree of anemia. In contrast acutely bled control mice with normal renal function showed a significant inverse correlation between the serum EPO level and hemoglobin concentration, indicating an appropriate response to anemia. The chronic administration of recombinant human EPO raised the hemoglobin concentration of CRF mice, a therapeutic effect which was independent of the initial degree of anemia. These observations suggest that this animal model has wide applicability for the study of anemia secondary to CRF.

Introduction

Anemia is present in the majority of patients with severe chronic renal failure (CRF) [1, 2]. The anemia results in many clinical disturbances leading not infrequently to major organ dysfunction, involving the heart in particular. In the absence of complicating factors, the anemia is primarily due to a relative deficiency of erythropoietin (EPO) production by the diseased kidneys [3–5]. Since the availability of recombinant human EPO (r-HuEPO) the anemia of large numbers of patients with advanced CRF has been successfully treated whether before or after the start of renal replacement therapy [3–6].

The anemia of CRF has been studied previously in many animal species, particularly the rat [7–9]. Because of the lack of a reproducible mouse model of severe CRF, the anemia of CRF has never been examined in the mouse, a species which has been used extensively previously for the study of anemia of various other etiologies [10]. There are several potential advantages to the use of mice for such studies including the availability of well-defined inbred mouse strains, the relative low cost of purchase and maintenance of mice compared to larger animal species, and the possibility of conducting large experiments with animals easy to maintain under standard conditions of husbandry. A prior limitation in using mice with small blood volumes has been obviated by the introduction of microassay methods requiring only small test samples.

We recently reported a model of surgically-induced renal failure in mice [11], which over time developed the main features of CRF such as growth retardation, bone disease, lipid abnormalities and anemia [12–14]. This

KARGER
E-Mail karger@karger.ch
Fax + 41 61 306 12 34

0028–2766/96/0724–0654$10.00/0

Dr. Raymonde F. Gagnon
Division of Nephrology
2nd Floor, Livingston Hall, Montreal General Hospital
1650 Cedar Avenue
Montreal, Que. H3G 1A4 (Canada)

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paper presents a detailed characterization of the anemia in CRF mice including its response to treatment with r-HuEPO. The hematological features of normal mice rendered acutely anemic by controlled bleeding are presented for comparison.

Materials and Methods

Animals

Five-week-old C57BL/6J female mice were purchased from Canadian Breeding (St. Constant, Que.) and left to acclimatize for 1 week prior to use. The mice were housed in an all-purpose animal facility and provided with standard mouse food in pellet form (Ralston Purina Co., St. Louis, Mo., USA) containing approximately 20% protein by weight with free access to untreated tap water ad libitum.

Production of Renal Failure

Renal failure was induced by a two-step procedure involving electrocoagulation of the surface of the surgically exposed right kidney and left nephrectomy. Details of this method have been reported previously [11]. Briefly, electrocoagulation of the entire surface of the right kidney except for a 2-mm rim of renal tissue around the hilum was followed by left nephrectomy 2 weeks later. All animals were subjected to electrocoagulation of the kidney surface or nephrectomy under controlled ether anesthesia with surgical approach through small bilateral flank incisions leaving the intestines and the upper abdominal content undisturbed. Renal electrocoagulation was performed using a foot-operated single point cauterizer angled at 30° (Hyfrecator, Model X-712, Birtcher Corp., Los Angeles, Calif., USA). The right kidney was freed from perirenal fat and the adrenal gland prior to electrocoagulation and special care was taken not to mobilize and injure the ureter; after electrocoagulation, the kidney was replaced into the renal fossa and completely covered by the tissue of the abdominal wall and skin. Two weeks later, a left nephrectomy was performed under identical operating conditions. After each surgical procedure, the incisions were closed in layers with clips applied to the skin. The duration of surgery from skin-to-skin never exceeded 5 min. Unless stated otherwise, the animals were studied 6 weeks after the second surgical procedure (left nephrectomy). The degree of renal failure was defined by the blood urea nitrogen (BUN) concentration measured at sacrifice.

Induction of Acute Anemia

Anemia was induced acutely in normal mice by controlled bloodletting under general anesthesia with carbon dioxide. Using a Pasteur pipette, a small volume of blood (0.4 ml) was collected from the retro-orbital venous plexus. This procedure was repeated on 3 successive days and the phlebotomized mice were studied on the 4th day at the time of sacrifice. In preliminary studies, this approach was shown to induce a reproducible, severe anemia.

Blood Collection and Processing

Blood was drawn either from the retro-orbital venous plexus using a Pasteur pipette or by transthoracic cardiac puncture using a 1-ml syringe. A 0.3-ml volume for cellular hematological determinations was immediately transferred to an EDTA(K3)-containing tube to prevent clotting; the rest of the sample was processed to yield serum which was stored at –20°C until use.

Analyses of Circulating Cells

Routine hematological assessment was done by Coulter Counter (Model STKS, Coulter Diagnostics Inc., Hialeah, Fla., USA). Differential leukocyte counts were performed on the basis of 100 cells/slide on Wright-stained blood smears. Reticulocyte counts were performed on thick blood smears freshly prepared on glass slides and stained with brilliant cresyl blue.

Serum Assays

The stored serum samples of the mice were assayed for BUN, iron indices and EPO levels. The BUN concentration was measured using a commercial kit (Sigma Procedure No. 535) based on the colorimetric method of Crocker [15]. The serum iron indices (iron concentration, unsaturated and total iron binding capacity) were measured as part of the large biochemical testing performed by autoanalyzer (IL9 Autoanalyzer, Instrumentation Laboratory Inc., Lexington, Mass., USA). The serum ferritin levels were measured enzymatically following the procedure of the Ferritin Fluorometric Enzyme Immunoassay (Stratus®, Baxter Diagnostic Inc., Deerfield, Ill., USA) and read by the Stratus® Fluorometric Analyzer. Serum EPO levels were measured by two different commercial radioimmunoassays designed for the quantitative measurement of human serum EPO (Diagnostic Systems Laboratories Inc., cat. No. DSL1100, and EPO Trac™, Incstar Corp, Stillwater, Minn., USA). All mouse serum samples were assayed in duplicate together with the recommended standards and controls.

Bone Marrow Assessment

The morphology of the bone marrow was examined on transverse sections of the femur obtained at the level of the mid-shaft. The samples were processed according to standard histological procedures which included sequential decalcification and staining with the May-Grünwald-Giemsa stain. The microscopic examination of the bone marrow was performed according to the standard protocol used in man. The presence of cellular iron in the form of hemosiderin was sought in the bone marrow after staining by the Prussian blue reaction. Iron was identified within tissue macrophages as a greenish blue precipitate.

Treatment with Erythropoietin

A commercial injectable formulation of r-HuEPO (Eprex®, Ortho Pharmaceutical, 4,000 units/ml) was used for this study. The experimental protocol is outlined in table 1. Briefly, r-HuEPO was administered to CRF mice for 3 weeks starting 3 weeks after the onset of renal failure (time of the left nephrectomy). The treatment consisted of thrice weekly intraperitoneal injections of r-HuEPO freshly prepared in 0.5 ml sterile saline. Four different dosages of r-HuEPO were chosen (1, 2, 5 and 10 units/mouse) and saline alone was given to a 5th group of mice. Before the start of treatment, the CRF mice were randomized into 5 experimental groups (4 treatment groups and 1 untreated control group) according to their hemoglobin concentration.

Statistical Analysis

The data are reported as the mean and standard deviation with significance levels of $p < 0.05$ and $p < 0.01$. When not otherwise mentioned, the data sets were compared using the unpaired two tailed Student's t test. In 1 experiment (r-HuEPO treatment) paired t test analysis of the data (before and after treatment) was conducted. Correlation and regression analyses were applied to selected parameters and to the dose-response curve to r-HuEPO treatment. The F test was used to determine the significance of the correlations.

Results

Hematological Characteristics in the Normal Mouse

The results of the hematological tests performed on the peripheral blood from adult female mice of the C57BL/6J inbred strain are presented in table 2. A number of hematological species differences were found in the peripheral blood from normal mice when compared to reference values for healthy adult women [16]. The most notable differences consisted in the smaller red cell volume (mean corpuscular volume, MCV, of 45.52 versus 90 fl, respectively), the higher lymphocyte number (mean percent of circulating WBC of 92.97 versus 36.1, respectively) and the higher platelet count (mean count of 801 versus 295 × 10^9/l, respectively).

Hematological Characterization of the Mouse Model of CRF

The results of the hematological tests performed on the peripheral blood of normal and CRF mice are presented in table 3. Results obtained in phlebotomized mice are presented for the purpose of comparison. Mice with CRF presented marked hematological changes in the peripheral blood predominantly affecting the red cell series. The CRF mice developed a significant anemia with hemoglobin concentration, hematocrit and red blood cell count all significantly decreased compared to normal animals. The anemia was directly correlated with the degree of renal impairment as measured by the BUN values (fig. 1). Although the MCV was significantly decreased, the mean corpuscular hemoglobin (MCH) was not, and thus the mean corpuscular hemoglobin concentration (MCHC) was increased. The red cell distribution width (RDW) in CRF mice was narrower than in normal animals. The anemia in CRF mice was not associated with an increase in reticulocyte counts (table 3; fig. 2). The serum EPO levels of normal (15.05 ± 4.76 mU/ml) and CRF (20.41 ± 8.93 mU/ml) mice were not significantly different (table 3; fig. 3).

The CRF mice had significantly higher circulating white blood cell (WBC) counts than the normal mice with a decreased proportion of lymphocytes and a corresponding increased proportion of neutrophils (table 3). CRF did not affect the number, mean volume, and width of distribution of platelets.

The effect of CRF on serum iron indices was also investigated (table 4). The serum iron concentration was not altered by CRF. There was a significant proportional increase in total and unsaturated iron-binding capacity in CRF mice, thus the iron saturation which is calculated from these two indices was unchanged. The serum ferritin

Table 1. Experimental protocol for the therapeutic evaluation of r-HuEPO in CRF mice

Time, weeks	Events
0	Arrival of mice in animal facilities
0–1	Acclimatization
1	Electrocoagulation of right kidney surface
3	Left nephrectomy
6	Blood collection for hemoglobin determination
6–9	Treatment with r-HuEPO (thrice weekly 0.5 ml i.p. injections)
9	Sacrifice and evaluation

Female C57BL/6J inbred mice aged 5 weeks at the beginning of experimentation.

Table 2. Comparison of hematological parameters in normal mouse and man

Parameters	Normal mouse	Normal range in man
Hemoglobin, g/l	135.85 ± 8.37	120–160
HCT, %	38.75 ± 1.8	37–47
RBC, 10^{12}/l	8.66 ± 0.32	4.2–5.4
MCV, fl	45.52 ± 1.07	82–100
MCH, pg	16.31 ± 0.38	27–31
MCHC, g/l	358.03 ± 12.29	320–360
RDW, %	38.18 ± 1.58	12.7–16.0
Reticulocytes, %	2.33 ± 0.83	0.8–4.0
EPO, mU/ml	15.05 ± 4.76	10–16
WBC, 10^9/l	4.99 ± 2.34	4.8–10.8
Lymphocytes, %	92.97 ± 5.25	22.3–49.9
Neutrophils, %	6.68 ± 4.87	45.5–72.1
Monocytes, %	1 ± 1.15	0.7–7.5
Platelets, 10^5/l	801.61 ± 199.53	150–440
MPV, fl	5.75 ± 0.85	6.8–10
PDW, %	16.33 ± 1.08	12–16

Results of routine hematological testing performed by autoanalyzer in 13-week-old normal female C57BL/6J mice compared to reference range of values for healthy adult women [16]. Reticulocyte counts and WBC differential counts obtained from fresh blood smear preparations. Serum EPO levels measured by RIA. Data in mice represent mean ± SD with the number of determinations indicated in table 3.

HCT = Hematocrit; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red blood cell distribution width; WBC = white blood cells; MPV = mean platelet volume; PDW = platelet distribution width.

level, used as an index of iron stores, was unaffected by CRF. Of note there was an important species difference for serum ferritin between the normal mouse and man.

In order to better assess red cell production and morphology as well as quantification of iron stores, histological sections of bone marrow were obtained from normal and CRF mice. Results of Giemsa staining revealed a slight reduction in red cell production in CRF mice when compared to normal animals (results not shown). CRF did not influence iron stores which were noted to be abundant in the bone marrow.

Anemia of Acute Bleeding

Mice with acute anemia secondary to repeated phlebotomy, which were used as control animals for the anemia of CRF, demonstrated striking hematological abnormalities (table 3). The selected protocol of controlled bleeding caused profound anemia in the mice with a sig-

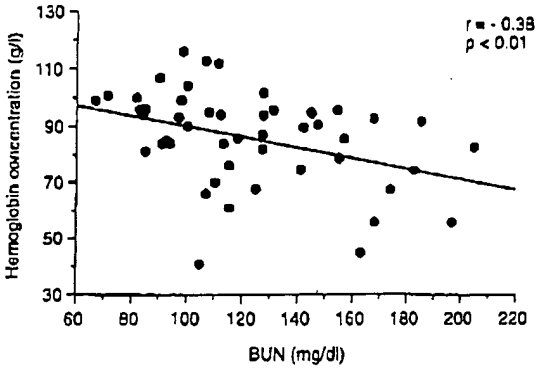


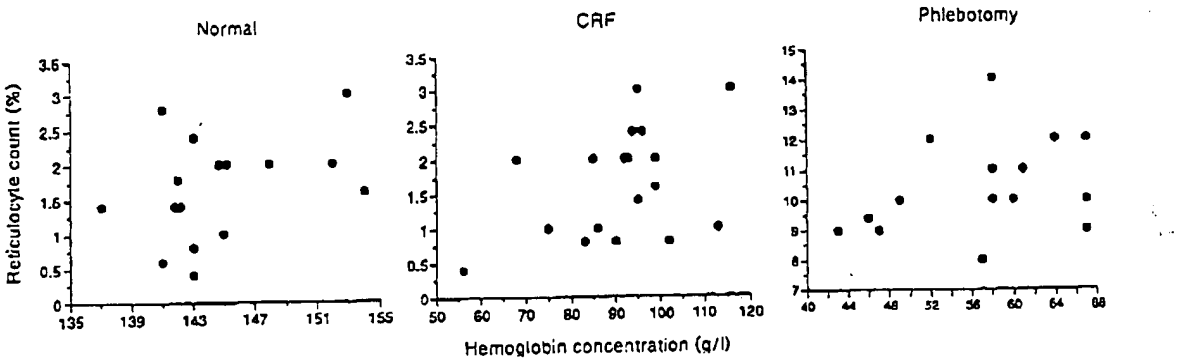
Fig. 1. Inverse correlation between hemoglobin concentration and BUN level in 13-week-old female C57BL/6J mice 6 weeks after the onset of renal failure (n = 47).

Table 3. Comparison of hematological parameters between normal, CRF and phlebotomized mice (mean ± SD)

Parameters	Normal		CRF		Phlebotomy	
BUN, mg/dl	22.28±5.97	(n = 29)	110.88±25.08**	(n = 31)	17.64±2.95	(n = 5)
Hemoglobin, g/l	135.85±8.37	(n = 39)	93.92±20.14**	(n = 66)	43.93±8.13**	(n = 14)
HCT, %	38.75±1.8	(n = 13)	25.4±6.32**	(n = 41)	12.45±2.71**	(n = 14)
RBC, 10^{12}/l	8.66±0.32	(n = 13)	5.95±1.41**	(n = 41)	2.34±0.47**	(n = 41)
MCV, fl	45.52±1.07	(n = 39)	43.21±1.23**	(n = 66)	52.93±2.29**	(n = 14)
MCH, pg	16.31±0.38	(n = 38)	16.38±1.07	(n = 63)	18.81±0.59**	(n = 14)
MCHC, g/l	358.03±12.29	(n = 38)	379.16±32.74**	(n = 63)	355.93±20.1	(n = 14)
RDW, %	38.18±1.58	(n = 13)	34.22±2.73**	(n = 41)	42.07±2.52**	(n = 14)
Reticulocytes, %	2.33±0.83	(n = 8)	2.68±1.27	(n = 10)	15.89±4.17**	(n = 9)
EPO, mU/ml	15.05±4.76	(n = 10)	20.41±8.93	(n = 11)	150.62±56.7**	(n = 8)
WBC, 10^9/l	4.99±2.34	(n = 39)	8.88±5.95**	(n = 66)	11.54±6.45**	(n = 13)
Lymphocytes, %	92.97±5.25	(n = 38)	90.37±6.13*	(n = 41)	71.17±11.99**	(n = 12)
Neutrophils, %	6.68±4.87	(n = 38)	8.98±5.82	(n = 41)	28.17±12.01**	(n = 12)
Monocytes, %	1±1.15	(n = 13)	1.59±1.46	(n = 17)	0.67±0.49	(n = 12)
Platelets, 10^9/l	801.61±199.53	(n = 36)	774.75±252.77	(n = 65)	837±122.59	(n = 14)
MPV, fl	5.75±0.85	(n = 13)	5.82±0.6	(n = 41)	5.79±0.37	(n = 14)
PDW, %	16.33±1.08	(n = 13)	16.26±0.77	(n = 41)	16.37±0.74	(n = 14)

Results of routine hematological testing in 13-week-old female C57BL/6J mice at the time of sacrifice 6 weeks after the onset of renal failure (CRF), in mice with normal renal function made acutely anemic by controlled bleeding (phlebotomy) and in normal control littermates. Number of determinations in each group indicated in parentheses. Reticulocyte counts and WBC differential counts obtained from fresh blood smear preparations. Serum EPO levels measured by RIA.

Significant differences between the 2 experimental animal groups and normal control mice:
* $p < 0.05$; ** $p < 0.01$. Abbreviations as in the legend to table 2.



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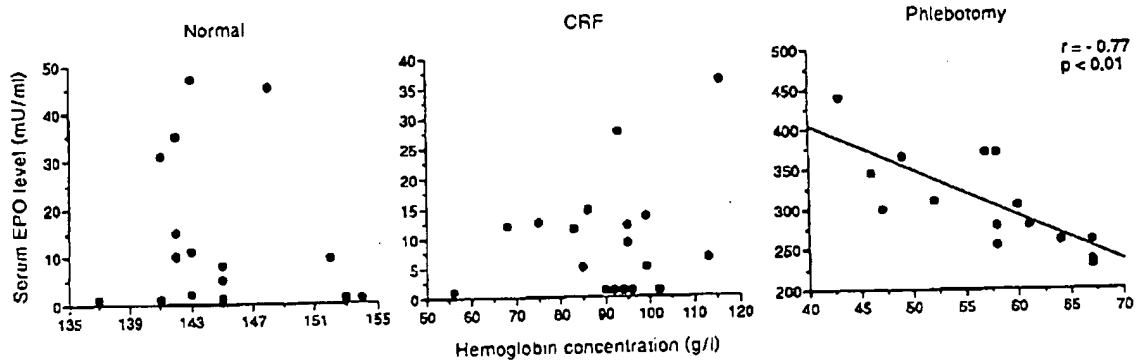

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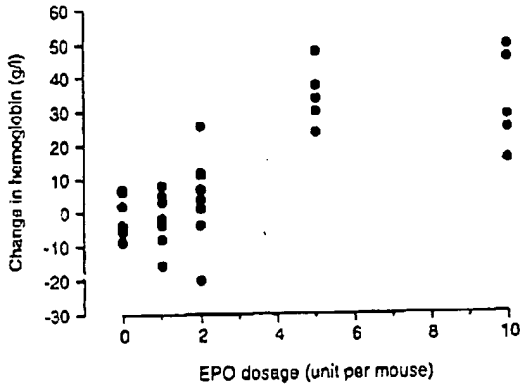
Fig. 2. Relationship between circulating reticulocyte count and hemoglobin concentration in 13-week-old female C57BL/6J mice 6 weeks after the onset of renal failure (CRF, n = 18), in mice with normal renal function made acutely anemic by controlled bleeding (phlebotomy, n = 15) and in normal control littermates (n = 14). Note the difference in scale of both axes between the animal groups.

Fig. 3. Relationship between serum EPO level and hemoglobin concentration in 13-week-old female C57BL/6J mice 6 weeks after the onset of renal failure (CRF, n = 18), in mice with normal renal function made acutely anemic by controlled bleeding (phlebotomy, n = 15) and in normal control littermates (n = 16). An inverse correlation is observed for the phlebotomized mice only. Note the difference in scale of both axes between the animal groups.

Fig. 4. The effect of r-HuEPO treatment on hemoglobin concentration in 13-week-old female C57BL/6J mice 6 weeks after the onset of renal failure (n = 32). The treatment was given for 3 weeks starting 3 weeks after the onset of renal failure. Treatment groups received intraperitoneal injections of 1, 2, 5 or 10 units of r-HuEPO thrice weekly. Untreated control CRF mice ('0' group on the left) received injections of saline only. Data represent the absolute change in hemoglobin concentration during the treatment period. The hemoglobin concentrations at the start of treatment were not significantly different between the 5 groups.



nificant fall in hemoglobin concentration, hematocrit and red blood cell number. The MCV, MCH and RDW values significantly increased, but the MCHC remained unchanged. The markedly anemic phlebotomized mice promptly developed a 6- to 8-fold increase in circulating reticulocytes (table 3; fig. 2). This increase was paralleled by a marked increase in serum EPO levels (10- to 20-fold) which was directly related to the degree of the anemia (table 3; fig. 3). The WBC count was also significantly increased in phlebotomized mice in association with a significant increase in the proportion of circulating neutrophils, presumably due to release of marginating neutrophils and early forms from the bone marrow. The platelet indices (platelet counts, mean platelet volume and platelet distribution) were not affected by the acute bleeding.

Table 4. Comparison of serum iron indices in normal and CRF mice (mean ± SD)

Parameters	Normal (n = 14)	CRF (n = 17)	Normal range in man
Iron, µmol/l	14.69 ± 4.8	17 ± 3.64	11–30
UIBC, µmol/l	46.54 ± 6.94	53.93 ± 6.69**	23–67
TIBC, µmol/l	61.23 ± 5.23	70.93 ± 6.6**	45–77
Iron saturation[1]	0.39 ± 0.2	0.39 ± 0.2	0.2–0.55
Ferritin, µg/l	1.9 ± 0.62	1.67 ± 1.02	10–200

Results of biochemical testing performed by autoanalyzer in 13-week-old female C57BL/6J mice at the time of sacrifice 6 weeks after the onset of renal failure (CRF) and in normal control littermates. Serum ferritin levels measured by fluorometric enzyme immunoassay. Reference range values for healthy adult women are presented for comparison [16].

Significant difference between the 2 animal groups: ** $p < 0.01$.

UIBC = Unsaturated iron-binding capacity; TIBC = total iron-binding capacity.

[1] Iron saturation expressed as the calculated fraction of total.

Treatment of the Anemia of CRF with r-HuEPO

The effectiveness of treatment with r-HuEPO to correct the anemia secondary to CRF was examined. The response of CRF mice to treatment with different doses of r-HuEPO is presented in table 5 and figure 4. Untreated CRF control mice did not show a significant change in hemoglobin concentration during the 3-week treatment period. Similarly, treatment with the lower r-HuEPO doses (1 and 2 units/mouse) did not appear to have a significant effect on the hemoglobin concentrations and WBC counts. The platelet counts, however, which varied widely between animals were significantly elevated at the end of the treatment period. Treatment with the higher r-HuEPO doses (5 and 10 units/mouse) produced significant increases in hemoglobin concentrations in CRF mice, without affecting the WBC and platelet counts. The therapeutic effect of r-HuEPO was present irrespective of the initial degree of anemia. There was a linear correlation ($r = 0.76$, $p < 0.01$) between the dosage of r-HuEPO and the absolute rise in hemoglobin concentration (fig. 4). The relatively 'low' r factor was due to the response to treatment apparently in three phases with the significant change occurring between the 1- and 5-units/mouse doses. Counts of circulating reticulocytes which were determined only at the end of the treatment period were significantly higher in the r-HuEPO-responsive mice when compared to both r-HuEPO-unresponsive mice and untreated control animals (data not shown).

Table 5. Hematological response to r-HuEPO treatment in CRF mice (mean ± SD)

Parameters		Dose of r-HuEPO, units/mouse				
		0 (n = 7)	1 (n = 7)	2 (n = 8)	5 (n = 5)	10 (n = 5)
Hemoglobin, g/l	Before	108.43 ± 14.92	114.29 ± 17.03	112 ± 11.59	104.2 ± 24.65	103.2 ± 15.51
	After	107.14 ± 17.46	112.29 ± 21.47	116.62 ± 20.49	139 ± 19.84**	136.4 ± 23.43**
WBC, 10^9/l	Before	13.9 ± 3.08	13.57 ± 2.87	11.85 ± 2.89	10.41 ± 4.34	11.7 ± 3.1
	After	16.48 ± 4.22	13.53 ± 2.61	12.22 ± 4.75	11.17 ± 4.78	10.96 ± 1.53
Platelets, 10^9/l	Before	573 ± 190	533 ± 258	591 ± 119	460 ± 144	593 ± 206
	After	658 ± 382	911 ± 170**	760 ± 203*	565 ± 287	805 ± 278

Results of routine hematological testing in female C57BL/6J mice 3 weeks (before treatment) and 6 weeks (after treatment) after the onset of renal failure at 7 weeks of age. Details of the treatment regime given in the Materials and Methods section.

Significant differences between before and after treatment values within each animal groups:
* $p < 0.05$; ** $p < 0.01$.

Discussion

Anemia is one of the main and most consistent clinical manifestations of CRF. Seventy-five percent of end-stage renal disease patients have a hematocrit level of less than 30% [1, 2]. Anemia develops when the creatinine clearance has decreased to 30–40 ml/min/1.73 m^2, and its severity increases with further deterioration of excretory renal function. Anemia in CRF is normochromic and normocytic when no other aggravating factors such as iron deficiency or aluminum overload coexist with the renal insufficiency. The anemia of renal failure is characteristically hyporegenerative with an inappropriate low reticulocyte response. The impact of anemia in CRF on physical and mental abilities is considerable and represents a major obstacle for the rehabilitation of patients with end-stage renal disease.

The inadequate production of EPO is the primary etiologic factor of anemia in CRF. This inadequate production of EPO results from the progressive destruction of renal production sites of EPO by the underlying renal disease. In comparison to anemic patients without renal disease, patients with anemia of renal failure display an inadequate rise in the serum concentration of EPO [1–6, 17–19]. The importance of EPO in the anemia of CRF has also been demonstrated in the clinical situation by the remarkable rise in hemoglobin following administration of r-HuEPO to patients with end-stage renal disease undergoing regular hemodialysis [3–6].

The mice with CRF demonstrated the salient hematological features observed in man with severely impaired renal function [12–14]. Anemia usually progresses as renal failure worsens and, indeed, a statistically significant correlation was found between the BUN level and the degree of anemia in the CRF mice. As in man with CRF, the lack of sufficient EPO was by far the most important of the anemia-causing factors, and consequently, the hypoproliferative features of the anemia tended to predominate. The erythrocytes appeared normal on blood smears and the reticulocyte count was within normal limits. The leukocyte count was usually normal but slight neutrophilic leukocytosis was observed in a single experiment, as is occasionally seen clinically. The platelet count was normal. The bone marrow was moderately hypercellular with slight erythroid hypoplasia, but erythrocyte maturation appeared morphologically normal. As is the case in man with CRF, in the absence of complicating factors, the values for serum iron were normal with abundant iron stores demonstrated in the bone marrow. These results were not unexpected as the anemia associated with CRF is not due primarily to iron deficiency but rather to a relative deficiency in EPO production by the diseased kidney. These hematological changes of hyporegenerative anemia in the presence of ample iron stores are typical of the uncomplicated anemia associated with severe CRF in man.

The present study confirmed that serum EPO levels are unchanged in CRF [1–6, 17–19]. The serum EPO levels were measured by two commercial RIA assays originally developed for determinations in man using an anti-human EPO antibody known to cross-react with rat EPO. This study has established that these two antibodies also cross-react with mouse EPO. The serum EPO levels in the normal mouse were comparable to that of man. Furthermore the results obtained in normal and phlebotomized mice were comparable with published values [20]. As expected phlebotomized mice demonstrated a significant rise in serum EPO levels which was linearly related to the degree of anemia. In contrast, the serum EPO levels observed in CRF mice were unchanged. Furthermore, the usual negative correlation between serum EPO levels and hemoglobin concentrations (as seen in the phlebotomized mice) was lost in CRF mice, an observation that is indicative of the relative failure of the normal feedback control mechanisms. This investigation, however, did not specifically address whether any feedback control was preserved, for instance if even the very low levels of EPO production in CRF changed in response to hemorrhage or transfusion.

The unchanged serum EPO levels in CRF mice were inappropriately low for the degree of anemia. It remains unclear why these normal serum EPO levels are unable to prevent the anemia. A possible explanation may lie in one of the known limitations of all radioimmunoassays used in the measurement of EPO in that they detect immunoreactive but not necessarily bioactive hormone. Thus, in renal failure, when serum EPO levels are low or undetectable, the radioimmunoassay detects higher levels than those detected by the traditional polycythemia mouse assay. Immunologically reactive EPO fragments that are biologically inactive may account for this difference.

The hematological effects of repeated phlebotomy in normal mice were examined in the present study. The observed changes are characteristic of acute posthemorrhagic anemia which has reached the stage of erythrocyte regeneration as indicated by the presence of a high degree reticulocytosis and macrocytosis [2]. Furthermore a neutrophilic leukocytosis was observed, explained in part by the effect of epinephrine on the mobilization of granulocytes from the marginal pool and also by their release from the marrow granulocytic reserve.

r-HuEPO became available for the treatment of anemia of end-stage renal disease in 1989 [6]. Therapy with this agent is effective and safe, and it substantially improves the quality of life. Although costly, it must now be considered the treatment of choice. The initial dose of r-HuEPO ranges from 50 to 150 units/kg three times per week, but subsequent adjustments in increments of 12.5–25 units/kg are necessary to individualize the dose. Target hematocrit levels of 33–38% have been recommended. Maintenance doses vary widely (12.5–525 units/kg) but more than 80% of patients require less than 150 units/kg. Ordinarily, the hormone is administered as an intravenous bolus, but it is also effective when given subcutaneously. Over 95% of patients respond to treatment and those who do not have a complicating, additional cause of anemia. On average, the hematocrit increases from 22 to over 35% within 12 weeks.

The effect of r-HuEPO on hemoglobin concentration was investigated in CRF mice. The usual recommended starting doses of r-HuEPO in man of 50–150 units/kg corresponded to doses of 1–3 U in mice with an average body weight of 20 g. Doses of 1, 2, 5 and 10 units/mouse were used in this experiment. The response of the CRF mice to the treatment strongly suggests a S-shaped biological curve. The lag phase of no response to the lower doses of r-HuEPO (1 and 2 units/mouse) argues that there is a biological mechanism which ignores low EPO doses but triggers erythropoiesis when a critical EPO level is reached. A major response to the treatment was observed at the higher r-HuEPO doses (5 and 10 units/mouse). The absence of difference of the response between 5 and 10 units/mouse of r-HuEPO is indicative of physiological saturation, suggesting that no further therapeutic gain is achieved by the highest dose of 10 units/mouse, which is an important consideration in treating the anemia of CRF.

The present study describes a mouse model which duplicates the hematological features of the anemia of CRF, including its response to r-HuEPO. The availability of this model will enable further investigation of the anemia secondary to CRF and should bring a better understanding of the pathogenesis and treatment of this obligatory consequence of CRF.

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